EAGER IMPRESS-U:
High Throughput Agile Interfaces for Cell Sorting (HiTACS)

The aging of the population, genetic abnormalities and other diseases, as well as extensive military conflicts raise serious challenges for effective regenerative and organ-substituting medicine. Modern regenerative medicine requires massive production of specific therapeutic cells to cover or fill in a defect in a damaged area up to the need to rebuild the entire damaged organ or its parts. A revolutionary progress in the field came with the discovery of the procedure of adult cell reprogramming resulting in induced pluripotent stem (iPS) cell generation. Upon proper, highly controlled guiding iPS cells can potentially develop into every cell type and form organs or even human embryos. Required target cells and cell types with healing properties and capabilities should be quickly and easily grown in sufficient amounts. However, the most important problem is to effectively select therapeutic cells out of other cells, especially of potentially dangerous, damaged, or transformed (tumorigenic) ones. The major sorting methods of live or dead mammalian and microbial cells and viruses use the unique recognition mechanism of antibodies. The antibody can recognize specific motifs within the molecules (proteins, glyco/lipo-proteins, polysaccharides) exposed on the cell membrane or extracellular matrix and bind to the surface of the cell. This mechanism is used to label cells with fluorescent dyes or attach them to magnetic beads covered with the antibody. The two sorting methods, fluorescent-activated cell sorting (FACS) and immunomagnetic cell sorting (MACS), rely on highly specific antigen-antibody interactions. These methods are accurate but cannot discriminate between different levels of antigen expression, and the sorting can also be affected by nonspecific interactions. Also, these methods are expensive, can lead to mechanical damage and partial loss of target cells, and are not scalable for the level of large-volume cell manufacturing.

In this research program, we propose to boost cell desorption using dynamic interfaces of polymer brushes or polymer hydrogels aimed at high throughput systems. The force sufficient for cell desorption will be generated by osmosis at the interface that undergoes phase transition in aqueous media. Among the various mechanisms that can cause such phase transition, we have chosen the thermo-induced reversible phase separation in a polymer-water system, which occurs close to the cell culture temperature. By oscillating temperatures below and above the low critical solution temperature, we can alternate the polymer material between its swollen and condensed states.

The dynamic interactions of polymer interface with cells enable us to accomplish affinity-based sorting without the application of high-cost natural or synthetic antibodies, polypeptides, DNA, etc. Nonspecific affinity-based cell sorting is not known yet and falls into the category of high-risk, high-reward. If successful, the developed technology will provide a new opportunity to discriminate cells by expressed functional motifs, size, shape, and mechanical properties. This transformative technology will lead to cheap and easily scalable cell separation processes, potentially revealing the new phenotypes of cells that cannot be discriminated using currently available antibodies.

Goal: This high risk - high payoff research program aims to introduce, research, and explore a new concept of cell separation, sorting, and analysis without using antibodies and other kinds of expensive or even unavailable biological molecules for cell discrimination.

To validate our hypotheses and to achieve our primary goal we plan to perform such tasks:

1. To build up the theoretical and computer simulation models for the smart surfaces aimed on the adsorption/desorption of cells that contain a set of parameters affecting the microscopic and mesoscopic structure of polymer brush interface, the interactions between cells and polymers, shape and size of cells, temperature regimes.
2. To perform theoretical analysis and computer simulation studies of developed models, resulting in clarification of the optimal molecular architecture, density and spatial patterning for smart surface targeted on efficient selectivity of cells out the complex mixtures;
3. To synthesize poly(N-isopropylacrylamide) (PNIPAAm) based smart surfaces with precisely tuned superficial architecture, temperature, hydrophilicity and swelling that take into account the findings of theoretical studies and computer simulations; check their properties experimentally;
4. To validate and tune up the specificity and efficacy of generated smart surfaces sorting procedure using *in vitro* tissue culture mixtures of different fluorescently labelled cell types and to compare its efficiency with traditional MACS and FACS cell sorting.

This research goal aligns with the need to strengthen international interdisciplinary collaborations and involve collaborators from Ukraine and Poland in the joint research program with U.S. scholars and students. Our interactions will boost the efforts to educate new generations of scientists and engineers with state-of-the-art knowledge and skills, a global vision of societal problems, sustainable approaches, and core human values. The students and young researchers of the collaborating teams will benefit from the combination of interdisciplinary methods, expertise, and cultural and ethnic diversity.